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FILE 'WPINDEX' ACCESS NOT AUTHORIZED

=> file home

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	91.21	91.42

FILE 'HOME' ENTERED AT 13:07:01 ON 05 DEC 2006

=> File Caplus, Embase, Medline

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	0.42	91.84

FILE 'CAPLUS' ENTERED AT 13:08:24 ON 05 DEC 2006  
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FILE 'EMBASE' ENTERED AT 13:08:24 ON 05 DEC 2006  
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FILE 'MEDLINE' ENTERED AT 13:08:24 ON 05 DEC 2006

=> S Cysteine WITH (insertion OR Added) with (Variant OR Mutant OR Mutein)  
MISSING OPERATOR 'WITH (INSERTION'  
The search profile that was entered contains terms or  
nested terms that are not separated by a logical operator.

=> S Cysteine WITH (Insertion OR Added) WITH (Variant OR Mutant OR Mutein)  
MISSING OPERATOR 'WITH (INSERTION'  
The search profile that was entered contains terms or  
nested terms that are not separated by a logical operator.

=> S Cysteine WITH Added WITH (Variant OR Mutant OR Mutein)  
MISSING OPERATOR 'WITH (VARIANT'  
The search profile that was entered contains terms or  
nested terms that are not separated by a logical operator.

=> S cysteine WITH added  
L1 324 CYSTEINE WITH ADDED

=> S Cysteine INsert?  
L2 33 CYSTEINE INSERT?

=> S Variant OR Mutant OR Mutein  
L3 1025994 VARIANT OR MUTANT OR MUTEIN

=> S L1 AND L2  
L4 0 L1 AND L2

=> S L1 AND L3  
L5 10 L1 AND L3

=> S L2 AND L3  
L6 18 L2 AND L3

=> Dup Remove L5  
PROCESSING COMPLETED FOR L5  
L7 10 DUP REMOVE L5 (0 DUPLICATES REMOVED)

=> Dup Remove L6  
PROCESSING COMPLETED FOR L6  
L8 10 DUP REMOVE L6 (8 DUPLICATES REMOVED)

=> D Ibib 15 1-10

L5 ANSWER 1 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN  
ACCESSION NUMBER: 2005:238537 CAPLUS  
DOCUMENT NUMBER: 142:310938  
TITLE: Fusion proteins composed of carotenoid-binding peptides and phenol oxidizing enzymes (phenol oxidase B or laccase), and their use in construction of detergent for bleaching carotenoid stain on fabric or surface  
INVENTOR(S): Wolfgang, Aehle; Baldwin, Toby M.; Van Gastel, Franciscus J. C.; Janssen, Giselle G.; Murray, Christopher J.; Wang, Huaming; Winetzky, Deborah S.  
PATENT ASSIGNEE(S): Neth.  
SOURCE: U.S. Pat. Appl. Publ., 107 pp.  
CODEN: USXXCO  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2005058996	A1	20050317	US 2002-235043	20020903
PRIORITY APPLN. INFO.:			US 2002-235043	20020903

L5 ANSWER 2 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN  
ACCESSION NUMBER: 2005:238410 CAPLUS  
DOCUMENT NUMBER: 142:291899  
TITLE: Cysteine variants of growth hormone and related proteins and their therapeutic uses  
INVENTOR(S): Cox, George N., III  
PATENT ASSIGNEE(S): USA  
SOURCE: U.S. Pat. Appl. Publ., 66 pp., Cont.-in-part of U.S. 6,753,165.  
CODEN: USXXCO  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 5

## PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2005058621	A1	20050317	US 2003-685288	20031013
WO 9903887	A1	19990128	WO 1998-US14497	19980713
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
WO 2000042175	A1	20000720	WO 2000-US931	20000114
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 6608183	B1	20030819	US 2000-462941	20000114
WO 2001087925	A2	20011122	WO 2001-US16088	20010516
WO 2001087925	A3	20020801		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
NZ 535718	A	20060929	NZ 2001-535718	20010516
US 6753165	B1	20040622	US 2001-889273	20010906
US 2003171284	A1	20030911	US 2002-298148	20021115
US 2003162949	A1	20030828	US 2003-400377	20030326
US 2004018586	A1	20040129	US 2003-276358	20030410
AU 2006203197	A1	20060817	AU 2006-203197	20060726
PRIORITY APPLN. INFO.:				
			US 1997-52516P	P 19970714
			WO 1998-US14497	W 19980713
			US 1999-116041P	P 19990114
			US 2000-462941	A3 20000114
			WO 2000-US931	W 20000114
			US 2000-204617P	P 20000516
			WO 2001-US16088	W 20010516
			US 2001-889273	A2 20010906
			US 2001-332285P	P 20011115
			US 2002-418040P	P 20021011
			US 2002-418105P	P 20021011
			US 2002-418106P	P 20021011
			US 2002-298148	A2 20021115
			US 2003-400377	A2 20030326
			US 2003-276358	A2 20030410
			NZ 2001-522847	A1 20010516
			AU 2002-306305	A3 20021128

L5 ANSWER 3 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2004:978332 CAPLUS

DOCUMENT NUMBER: 142:175381

TITLE: Modified interferon-beta, and chemically modified conjugates thereof

INVENTOR(S): Lee, Eun Jeong; Lee, Ji Won; Noh, Gwang; Park, Min Gu

PATENT ASSIGNEE(S): Sunbio Inc., S. Korea  
 SOURCE: Repub. Korean Kongkae Taeho Kongbo, No pp. given  
 CODEN: KRXXA7  
 DOCUMENT TYPE: Patent  
 LANGUAGE: Korean  
 FAMILY ACC. NUM. COUNT: 1  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
KR 2003037598	A	20030514	KR 2001-68852	20011106
PRIORITY APPLN. INFO.:			KR 2001-68852	20011106

L5 ANSWER 4 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN  
 ACCESSION NUMBER: 2004:845793 CAPLUS  
 DOCUMENT NUMBER: 142:73484  
 TITLE: L-threonine production by Serratia  
 INVENTOR(S): Choe, Inn Suk; Chun, Jun Hyun; Yu, Yong Soo  
 PATENT ASSIGNEE(S): Daesang Corporation, S. Korea  
 SOURCE: Repub. Korea, No pp. given  
 CODEN: KRXXFC  
 DOCUMENT TYPE: Patent  
 LANGUAGE: Korean  
 FAMILY ACC. NUM. COUNT: 1  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
KR 168720	B1	19990115	KR 1995-51142	19951218
PRIORITY APPLN. INFO.:			KR 1995-51142	19951218

L5 ANSWER 5 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN  
 ACCESSION NUMBER: 2004:601396 CAPLUS  
 DOCUMENT NUMBER: 141:273856  
 TITLE: Synthesis of fluorescent oligonucleotide-EYFP  
 conjugate: Towards supramolecular construction of  
 semisynthetic biomolecular antennae  
 AUTHOR(S): Kukolka, Florian; Niemeyer, Christof M.  
 CORPORATE SOURCE: Fachbereich Chemie Biologisch-Chemische  
 Mikrostrukturtechnik, Universitaet Dortmund, Dortmund,  
 D-44227, Germany  
 SOURCE: Organic & Biomolecular Chemistry (2004), 2(15),  
 2203-2206  
 CODEN: OBCRAK; ISSN: 1477-0520  
 PUBLISHER: Royal Society of Chemistry  
 DOCUMENT TYPE: Journal  
 LANGUAGE: English  
 REFERENCE COUNT: 22 THERE ARE 22 CITED REFERENCES AVAILABLE FOR THIS  
 RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 6 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN  
 ACCESSION NUMBER: 2002:561864 CAPLUS  
 DOCUMENT NUMBER: 137:321715  
 TITLE: Natural Supramolecular Building Blocks  
 Cysteine-Added Mutants of  
 Cowpea Mosaic Virus  
 AUTHOR(S): Wang, Qian; Lin, Tianwei; Johnson, John E.; Finn, M.  
 G.  
 CORPORATE SOURCE: Department of Chemistry and The Skaggs Institute for  
 Chemical Biology, The Scripps Research Institute, La  
 Jolla, CA, 92037, USA  
 SOURCE: Chemistry & Biology (2002), 9(7), 813-819  
 CODEN: CBOLE2; ISSN: 1074-5521  
 PUBLISHER: Cell Press  
 DOCUMENT TYPE: Journal

LANGUAGE: English  
REFERENCE COUNT: 14 THERE ARE 14 CITED REFERENCES AVAILABLE FOR THIS  
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 7 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN  
ACCESSION NUMBER: 1991:402487 CAPLUS  
DOCUMENT NUMBER: 115:2487  
TITLE: Cysteine-added variants  
of polypeptides and chemical modifications thereof  
INVENTOR(S): Shaw, Gray; Veldman, Geertruida; Wooters, Joseph  
PATENT ASSIGNEE(S): Genetics Institute, Inc., USA  
SOURCE: PCT Int. Appl., 47 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9012874	A2	19901101	WO 1990-US2144	19900419
WO 9012874	A3	19910110		
W: AU, CA, JP				
RW: AT, BE, CH, DE, DK, ES, FR, GB, IT, LU, NL, SE				
US 5166322	A	19921124	US 1989-341990	19890421
AU 9055537	A1	19901116	AU 1990-55537	19900419
EP 469074	A1	19920205	EP 1990-907849	19900419
EP 469074	B1	19960731		
R: AT, BE, CH, DE, DK, ES, FR, GB, IT, LI, LU, NL, SE				
JP 04504801	T2	19920827	JP 1990-507086	19900419
JP 2557144	B2	19961127		
EP 668353	A1	19950823	EP 1995-103989	19900419
R: AT, BE, CH, DE, DK, ES, FR, GB, IT, LI, LU, NL, SE				
EP 668354	A1	19950823	EP 1995-103990	19900419
R: AT, BE, CH, DE, DK, ES, FR, GB, IT, LI, LU, NL, SE				
AT 140969	E	19960815	AT 1990-907849	19900419
ES 2090132	T3	19961016	ES 1990-907849	19900419
PRIORITY APPLN. INFO.:			US 1989-341990	A 19890421
			EP 1990-907849	A3 19900419
			WO 1990-US2144	A 19900419

L5 ANSWER 8 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN  
ACCESSION NUMBER: 1983:483172 CAPLUS  
DOCUMENT NUMBER: 99:83172  
TITLE: Cysteine starvation, isoleucyl-tRNA<sup>Ile</sup>, and the  
regulation of the ilvGEDA operon of Escherichia coli  
AUTHOR(S): Harris, Charles L.; Lui, Lorena; Sakallah, Sameer;  
DeVore, Russell  
CORPORATE SOURCE: Sch. Med., West Virginia Univ., Morgantown, WV, 26506,  
USA  
SOURCE: Journal of Biological Chemistry (1983), 258(12),  
7676-83  
CODEN: JBCHA3; ISSN: 0021-9258  
DOCUMENT TYPE: Journal  
LANGUAGE: English

L5 ANSWER 9 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN  
ACCESSION NUMBER: 1981:99497 CAPLUS  
DOCUMENT NUMBER: 94:99497  
TITLE: Regulation of nitrite reductase in the denitrifying  
bacterium Alcaligenes faecalis S-6  
AUTHOR(S): Kakutani, Tetsu; Beppu, Teruhiko; Arima, Kei  
CORPORATE SOURCE: Fac. Agric., Univ. Tokyo, Tokyo, 113, Japan  
SOURCE: Agricultural and Biological Chemistry (1981), 45(1),  
23-8

CODEN: ABCHA6; ISSN: 0002-1369  
DOCUMENT TYPE: Journal  
LANGUAGE: English

L5 ANSWER 10 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN  
ACCESSION NUMBER: 1969:499239 CAPLUS  
DOCUMENT NUMBER: 71:99239  
TITLE: Delayed lysis with Salmonella bacteriophage P22:  
induction of lysis by addition of cysteine or  
histidine to the growth medium  
AUTHOR(S): Cohen, Larry William  
CORPORATE SOURCE: Pomona Coll., Claremont, CA, USA  
SOURCE: Journal of Virology (1969), 4(3), 214-18  
CODEN: JOVIAM; ISSN: 0022-538X  
DOCUMENT TYPE: Journal  
LANGUAGE: English

=> D Ibib L8 1-10

L8 ANSWER 1 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN  
ACCESSION NUMBER: 2005:1355291 CAPLUS  
DOCUMENT NUMBER: 144:47224  
TITLE: Heat stability of maize endosperm ADP-glucose  
pyrophosphorylase is enhanced by insertion of a  
cysteine in the N terminus of the small subunit  
AUTHOR(S): Linebarger, Carla R. Lyerly; Boehlein, Susan K.;  
Sewell, Aileen K.; Shaw, Janine; Hannah, L. Curtis  
CORPORATE SOURCE: Program in Plant Molecular and Cellular Biology and  
Horticultural Sciences, University of Florida,  
Gainesville, FL, 32610-0245, USA  
SOURCE: Plant Physiology (2005), 139(4), 1625-1634  
CODEN: PLPHAY; ISSN: 0032-0889  
PUBLISHER: American Society of Plant Biologists  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
REFERENCE COUNT: 33 THERE ARE 33 CITED REFERENCES AVAILABLE FOR THIS  
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 2 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN  
ACCESSION NUMBER: 2005:187544 CAPLUS  
TITLE: Detailed reactivity study of cysteine-  
inserted cowpea mosaic virus extracted from  
primary and secondary leaves  
AUTHOR(S): Alexander, Lisa A.; Wang, Qian  
CORPORATE SOURCE: Department of Chemistry and Biochemistry, University  
of South Carolina, Columbia, SC, 29208, USA  
SOURCE: Abstracts of Papers, 229th ACS National Meeting, San  
Diego, CA, United States, March 13-17, 2005 (2005),  
CHED-403. American Chemical Society: Washington, D.  
C.  
CODEN: 69GQMP  
DOCUMENT TYPE: Conference; Meeting Abstract  
LANGUAGE: English

L8 ANSWER 3 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN  
ACCESSION NUMBER: 2003:892083 CAPLUS  
DOCUMENT NUMBER: 139:376203  
TITLE: Construction of recombinant calcium-binding  
cysteine-inserted photoproteins and  
their conjugates with ligands for analytical use  
INVENTOR(S): Inouye, Satoshi  
PATENT ASSIGNEE(S): Chisso Corporation, Japan  
SOURCE: U.S. Pat. Appl. Publ., 15 pp.  
CODEN: USXXCO



DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003212259	A1	20031113	US 2003-400630	20030328
JP 2004000143	A2	20040108	JP 2003-17505	20030127
PRIORITY APPLN. INFO.:			JP 2002-93849	A 20020329
			JP 2002-17505	A 20030127

L8 ANSWER 4 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 1  
ACCESSION NUMBER: 2003:624906 CAPLUS  
DOCUMENT NUMBER: 140:187111  
TITLE: Monitoring manufacturing process yields, purity and stability of structural variants of PEGylated staphylokinase mutant SY161 by quantitative reverse-phase chromatography  
AUTHOR(S): Johnson, Catharine; Royal, Mabel; Moreadith, Randall; Bedu-Addo, Frank; Advant, Siddharth; Wan, Min; Conn, Greg  
CORPORATE SOURCE: Diosynth-RTP, Pharmaceutical Sciences, Diosynth-RTP, Cary, NC, 27513, USA  
SOURCE: Biomedical Chromatography (2003), 17(5), 335-344  
CODEN: BICHE2; ISSN: 0269-3879  
PUBLISHER: John Wiley & Sons Ltd.  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
REFERENCE COUNT: 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 5 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 2  
ACCESSION NUMBER: 2001:491013 CAPLUS  
DOCUMENT NUMBER: 135:208715  
TITLE: Ca<sup>2+</sup>- and cross-bridge-dependent changes in N- and C-terminal structure of troponin C in rat cardiac muscle  
AUTHOR(S): Martyn, Donald A.; Regnier, Michael; Xu, Daguang; Gordon, Albert M.  
CORPORATE SOURCE: Department of Bioengineering, University of Washington, Seattle, WA, 98195, USA  
SOURCE: Biophysical Journal (2001), 80(1), 360-370  
CODEN: BIOJAU; ISSN: 0006-3495  
PUBLISHER: Biophysical Society  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
REFERENCE COUNT: 45 THERE ARE 45 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 6 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN  
ACCESSION NUMBER: 2000:9476 CAPLUS  
DOCUMENT NUMBER: 132:147013  
TITLE: Defining proximity relationships in the tertiary structure of the dopamine transporter. Identification of a conserved glutamic acid as a third coordinate in the endogenous Zn<sup>2+</sup>-binding site  
AUTHOR(S): Loland, Claus Juul; Norregaard, Lene; Gether, Ulrik  
CORPORATE SOURCE: Division of Cellular and Molecular Physiology, Department of Medical Physiology 12.5, The Panum Institute, University of Copenhagen, Copenhagen, DK-2200, Den.  
SOURCE: Journal of Biological Chemistry (1999), 274(52), 36928-36934  
CODEN: JBCHA3; ISSN: 0021-9258

PUBLISHER: American Society for Biochemistry and Molecular  
Biology  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
REFERENCE COUNT: 49 THERE ARE 49 CITED REFERENCES AVAILABLE FOR THIS  
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 7 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 3  
ACCESSION NUMBER: 1999:368901 CAPLUS  
DOCUMENT NUMBER: 131:154416  
TITLE: Role of region C in regulation of the heat shock  
gene-specific sigma factor of Escherichia coli,  
 $\sigma_{32}$   
AUTHOR(S): Arsene, Florence; Tomoyasu, Toshifumi; Mogk, Axel;  
Schirra, Christiane; Schulze-Specking, Agnes; Bukau,  
Bernd  
CORPORATE SOURCE: Institut fur Biochemie und Molekularbiologie,  
Universitat Freiburg, Freiburg, D-79104, Germany  
SOURCE: Journal of Bacteriology (1999), 181(11), 3552-3561  
CODEN: JOBAAY; ISSN: 0021-9193  
PUBLISHER: American Society for Microbiology  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
REFERENCE COUNT: 45 THERE ARE 45 CITED REFERENCES AVAILABLE FOR THIS  
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 8 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 4  
ACCESSION NUMBER: 1992:402910 CAPLUS  
DOCUMENT NUMBER: 117:2910  
TITLE: The anticodon and discriminator base are major  
determinants of cysteine tRNA identity in vivo  
AUTHOR(S): Pallanck, Leo; Li, Shihong; Schulman, LaDonne H.  
CORPORATE SOURCE: Dep. Dev. Biol. Cancer, Albert Einstein Coll. Med.,  
Bronx, NY, 10461, USA  
SOURCE: Journal of Biological Chemistry (1992), 267(11),  
7221-3  
CODEN: JBCHA3; ISSN: 0021-9258  
DOCUMENT TYPE: Journal  
LANGUAGE: English

L8 ANSWER 9 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN  
ACCESSION NUMBER: 1987:614673 CAPLUS  
DOCUMENT NUMBER: 107:214673  
TITLE: Plasmid transfer, chromosome mobilization and genetic  
linkage in Rhodopseudomonas sphaeroides  
AUTHOR(S): Wu, Yongqiang; Yu, Baolin; Song, Hongyu  
CORPORATE SOURCE: Shanghai Inst. Plant Physiol., Acad. Sin., Shanghai,  
Peop. Rep. China  
SOURCE: Weishengwu Xuebao (1987), 27(3), 238-43  
CODEN: WSHPA8; ISSN: 0001-6209  
DOCUMENT TYPE: Journal  
LANGUAGE: Chinese

L8 ANSWER 10 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN  
ACCESSION NUMBER: 1986:1702 CAPLUS  
DOCUMENT NUMBER: 104:1702  
TITLE: Stable proteins, methods for their construction,  
plasmids and other DNA encoding them, cell cultures  
harboring such plasmids  
INVENTOR(S): Wetzel, Ronald Burnell  
PATENT ASSIGNEE(S): Genentech, Inc., USA  
SOURCE: Eur. Pat. Appl., 42 pp.  
CODEN: EPXXDW  
DOCUMENT TYPE: Patent  
LANGUAGE: English



FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 155832	A2	19850925	EP 1985-301829	19850315
EP 155832	A3	19870819		
R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE				
JP 60224489	A2	19851108	JP 1985-53207	19850316
PRIORITY APPLN. INFO.:			US 1984-590121	A 19840316
			US 1984-649448	A 19840911

=> Hold

HOLD IS NOT A RECOGNIZED COMMAND

The previous command name entered was not recognized by the system.  
For a list of commands available to you in the current file, enter  
"HELP COMMANDS" at an arrow prompt (=>).

=> DIS HIST

(FILE 'HOME' ENTERED AT 13:06:03 ON 05 DEC 2006)

FILE 'MEDLINE, EMBASE, ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE, AQUASCI, BIOENG, BIOSIS, BIOTECHDS, BIOTECHNO, CABA, CAPLUS, CEABA-VTB, CIN, CONFSCI, CROPB, CROPU, DDFB, DGENE, DISSABS, DRUGB, DRUGMONOG2, DRUGU, EMBAL, ESBIODBASE, ...' ENTERED AT 13:06:38 ON 05 DEC 2006

FILE 'HOME' ENTERED AT 13:07:01 ON 05 DEC 2006

FILE 'CAPLUS, EMBASE, MEDLINE' ENTERED AT 13:08:24 ON 05 DEC 2006

L1	324 S CYSTEINE WITH ADDED
L2	33 S CYSTEINE INSERT?
L3	1025994 S VARIANT OR MUTANT OR MUTEIN
L4	0 S L1 AND L2
L5	10 S L1 AND L3
L6	18 S L2 AND L3
L7	10 DUP REMOVE L5 (0 DUPLICATES REMOVED)
L8	10 DUP REMOVE L6 (8 DUPLICATES REMOVED)

=>

=>

Executing the logoff script...

=> LOG H

COST IN U.S. DOLLARS	SINCE FILE	TOTAL
	ENTRY	SESSION
FULL ESTIMATED COST	52.94	144.78

SESSION WILL BE HELD FOR 120 MINUTES  
STN INTERNATIONAL SESSION SUSPENDED AT 13:21:38 ON 05 DEC 2006

Connecting via Winsock to STN

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SESSION RESUMED IN FILE 'CAPLUS, EMBASE, MEDLINE' AT 13:26:15 ON 05 DEC 2006  
FILE 'CAPLUS' ENTERED AT 13:26:15 ON 05 DEC 2006  
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FILE 'MEDLINE' ENTERED AT 13:26:15 ON 05 DEC 2006

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	52.94	144.78

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(FILE 'HOME' ENTERED AT 13:06:03 ON 05 DEC 2006)

FILE 'MEDLINE, EMBASE, ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE, AQUASCI, BIOENG, BIOSIS, BIOTECHDS, BIOTECHNO, CABA, CAPLUS, CEABA-VTB, CIN, CONFSCI, CROPB, CROPU, DDFB, DGENE, DISSABS, DRUGB, DRUGMONOG2, DRUGU, EMBAL, ESBIODASE, ...' ENTERED AT 13:06:38 ON 05 DEC 2006

FILE 'HOME' ENTERED AT 13:07:01 ON 05 DEC 2006

FILE 'CAPLUS, EMBASE, MEDLINE' ENTERED AT 13:08:24 ON 05 DEC 2006

L1 324 S CYSTEINE WITH ADDED  
L2 33 S CYSTEINE INSERT?  
L3 1025994 S VARIANT OR MUTANT OR MUTEIN  
L4 0 S L1 AND L2  
L5 10 S L1 AND L3  
L6 18 S L2 AND L3  
L7 10 DUP REMOVE L5 (0 DUPLICATES REMOVED)  
L8 10 DUP REMOVE L6 (8 DUPLICATES REMOVED)

=> S L7 AND (N-terminus OR Amino-terminus)  
L9 2 L7 AND (N-TERMINUS OR AMINO-TERMINUS)

=> S L7 AND (C-terminus OR CooH-Terminus OR Carboxy-terminus)  
L10 2 L7 AND (C-TERMINUS OR COOH-TERMINUS OR CARBOXY-TERMINUS)

=> S L8 AND ((N-terminus OR Amino-terminus) OR ((C-TERMINUS OR COOH-TERMINUS OR CARBOXY-TERMINUS)

UNMATCHED LEFT PARENTHESIS 'OR ((C-TERMINU'

The number of right parentheses in a query must be equal to the number of left parentheses.

=> S L8 AND ((N-terminus OR Amino-terminus) OR (C-TERMINUS OR COOH-TERMINUS OR CARBOXY-TERMINUS))

L11 5 L8 AND ((N-TERMINUS OR AMINO-TERMINUS) OR (C-TERMINUS OR COOH-T  
ERMINUS OR CARBOXY-TERMINUS))

=> D Iall L9

L9 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2006 ACS on STN  
ACCESSION NUMBER: 2005:238537 CAPLUS  
DOCUMENT NUMBER: 142:310938  
ENTRY DATE: Entered STN: 18 Mar 2005  
TITLE: Fusion proteins composed of carotenoid-binding peptides and phenol oxidizing enzymes (phenol oxidase B or laccase), and their use in construction of detergent for bleaching carotenoid stain on fabric or surface  
INVENTOR(S): Wolfgang, Aehle; Baldwin, Toby M.; Van Gastel, Franciscus J. C.; Janssen, Giselle G.; Murray,

PATENT ASSIGNEE(S): Christopher J.; Wang, Huaming; Winetzky, Deborah S.  
 SOURCE: Neth.  
 U.S. Pat. Appl. Publ., 107 pp.  
 CODEN: USXXCO  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 INT. PATENT CLASSIF.:  
     MAIN: C12Q001-68  
     SECONDARY: C07H021-04; C12N009-02; C11D003-386  
 US PATENT CLASSIF.: 435006000; 435069100; 435189000; 435320100; 435325000;  
 536023200; 510320000  
 CLASSIFICATION: 3-2 (Biochemical Genetics)  
 Section cross-reference(s): 7, 10, 46  
 FAMILY ACC. NUM. COUNT: 1  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2005058996	A1	20050317	US 2002-235043	20020903
PRIORITY APPLN. INFO.:			US 2002-235043	20020903

PATENT CLASSIFICATION CODES:

PATENT NO.	CLASS	PATENT FAMILY CLASSIFICATION CODES
US 2005058996	ICM	C12Q001-68
	ICS	C07H021-04; C12N009-02; C11D003-386
	INCL	435006000; 435069100; 435189000; 435320100; 435325000; 536023200; 510320000
	IPCI	C12Q0001-68 [ICM,7]; C07H0021-04 [ICS,7]; C07H0021-00 [ICS,7,C*]; C12N0009-02 [ICS,7]; C11D0003-386 [ICS,7]; C11D0003-38 [ICS,7,C*]
	IPCR	C11D0003-38 [I,C*]; C11D0003-386 [I,A]; C12N0009-02 [I,C*]; C12N0009-02 [I,A]
	NCL	435/006.000; 435/069.100; 435/189.000; 435/320.100; 435/325.000; 510/320.000; 536/023.200
	ECLA	C11D003/386H; C12N009/02H3B2

ABSTRACT:

The invention provides numerous peptides composed of 7 or 12 amino acids that are able to bind to a carotenoid compound on a fabric. The invention relates said peptides: (a) may also contain a cysteine added to each end (defined as C-C derivs.); (b) comprise a repeatable motif; and (c) bind to tomato or paprika stains on cotton. The invention also provides chimeric proteins composed of said peptides linked to the C-terminus of a phenol oxidizing enzyme, such as laccase and/or Stachybotrys phenol oxidase B, and/or variants thereof. The invention further provides polynucleotides encoding said carotenoid-binding peptide-phenol oxidizing enzyme fusion proteins, and use of said polynucleotides in transforming host cells for recombinant production of fusion proteins. Still further, the invention provides for the use of said recombinant fusion proteins in construction of a detergent, wherein said detergent can be used to clean a carotenoid stain on a fabric and/or a surface contacting the stain. Finally, the invention provides the amino acid sequences of Stachybotrys chartarum phenol oxidase B and carotenoid-binding peptides. In the examples, the invention demonstrated that a fusion protein composed of Seq ID number 16 carotenoid-binding peptide and a phenol oxidase B variant (M254F/E346V/E348Q) bound to tomato stain on cotton better than the enzyme alone.

SUPPL. TERM: fusion protein carotenoid binding peptide phenol oxidizing enzyme; sequence phenol oxidase B Stachybotrys use fusion protein; detergent stain remover carotenoid binding peptide phenol oxidizing enzyme; laccase carotenoid binding peptide fusion detergent stain remover

INDEX TERM: Detergents  
 (bleaching; fusion proteins composed of carotenoid-binding peptides and phenol oxidizing enzymes, and their use in construction of detergent for bleaching

carotenoid stain on fabric or surface)

INDEX TERM: Fusion proteins (chimeric proteins)  
 ROLE: BPN (Biosynthetic preparation); PRP (Properties); TEM (Technical or engineered material use); BIOL (Biological study); PREP (Preparation); USES (Uses)  
 (carotenoid-binding peptide fused to phenol oxidizing enzyme; fusion proteins composed of carotenoid-binding peptides and phenol oxidizing enzymes, their polynucleotides and use in construction of detergent)

INDEX TERM: Peptides, properties  
 ROLE: BPN (Biosynthetic preparation); PRP (Properties); TEM (Technical or engineered material use); BIOL (Biological study); PREP (Preparation); USES (Uses)  
 (carotenoid-binding, fusion product with phenol oxidizing enzyme; fusion proteins composed of carotenoid-binding peptides and phenol oxidizing enzymes, and their use in construction of detergent)

INDEX TERM: Textiles  
 (cotton; fusion proteins composed of carotenoid-binding peptides and phenol oxidizing enzymes, and their ability to better bind tomato stains on cotton than enzymes alone)

INDEX TERM: Detergents  
 Surface  
 Textiles  
 (fusion proteins composed of carotenoid-binding peptides and phenol oxidizing enzymes, and their use in construction of detergent for bleaching carotenoid stain on fabric or surface)

INDEX TERM: Carotenes, processes  
 ROLE: REM (Removal or disposal); PROC (Process)  
 (fusion proteins composed of carotenoid-binding peptides and phenol oxidizing enzymes, and their use in construction of detergent for bleaching carotenoid stain on fabric or surface)

INDEX TERM: Polynucleotides  
 ROLE: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)  
 (fusion proteins composed of carotenoid-binding peptides and phenol oxidizing enzymes, their polynucleotides and use in construction of detergent for bleaching carotenoid stain on fabric or surface)

INDEX TERM: Capsicum annum annum  
 (longum group, paprika; peptides able to bind carotenoid compds., their ability to bind carotenoids found in tomato or paprika and use in construction of fusion proteins with Stachybotrys phenol oxidizing enzymes)

INDEX TERM: Plasmid vectors  
 (pGAPT; polynucleotides encoding fusion proteins composed of carotenoid-binding peptides and phenol oxidizing enzymes, their vectors and use in transforming host cells for recombinant protein production)

INDEX TERM: Lycopersicon esculentum  
 (peptides able to bind carotenoid compds., their ability to bind carotenoids found in tomato or paprika and use in construction of fusion proteins with Stachybotrys phenol oxidizing enzymes)

INDEX TERM: Repeat motifs (protein)  
 (peptides able to bind carotenoid compds., their protein motifs, sequences and use in construction of fusion proteins with Stachybotrys phenol oxidizing enzymes for creation of detergent)

INDEX TERM: Molecular cloning  
 (polynucleotides encoding fusion proteins composed of carotenoid-binding peptides and phenol oxidizing enzymes,

ROLE: BPN (Biosynthetic preparation); PRP (Properties); TEM  
(Technical or engineered material use); BIOL (Biological  
study); PREP (Preparation); USES (Uses)

(peptides able to bind carotenoid compds., their  
sequences and use in construction of fusion proteins with  
Stachybotrys phenol oxidizing enzymes for creation of  
detergent)

INDEX TERM: 502697-64-5 502697-65-6 847967-48-0 847967-49-1  
847967-50-4 847967-51-5 847967-52-6 847967-53-7  
847967-54-8 847967-55-9 847967-56-0 847967-57-1  
847967-58-2

ROLE: PRP (Properties)

(unclaimed sequence; fusion proteins composed of  
carotenoid-binding peptides and phenol oxidizing enzymes  
(phenol oxidase B or laccase), and their use in  
construction of detergent for bleaching carotenoid stain  
on fabric or surface)

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(FILE 'HOME' ENTERED AT 13:06:03 ON 05 DEC 2006)

FILE 'MEDLINE, EMBASE, ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR,  
ANTE, AQUALINE, AQUASCI, BIOENG, BIOSIS, BIOTECHDS, BIOTECHNO, CABA,  
CAPLUS, CEABA-VTB, CIN, CONFSCI, CROPB, CROPU, DDFB, DGENE, DISSABS,  
DRUGB, DRUGMONOG2, DRUGU, EMBAL, ESBIODASE, ...' ENTERED AT 13:06:38 ON  
05 DEC 2006

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L10 2 S L7 AND (C-TERMINUS OR COOH-TERMINUS OR CARBOXY-TERMINUS)  
L11 5 S L8 AND ((N-TERMINUS OR AMINO-TERMINUS) OR (C-TERMINUS OR CO

=> D Iall L9 2

L9 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1991:402487 CAPLUS

DOCUMENT NUMBER: 115:2487

ENTRY DATE: Entered STN: 12 Jul 1991

TITLE: Cysteine-added variants  
of polypeptides and chemical modifications thereof

INVENTOR(S): Shaw, Gray; Veldman, Geertruida; Wooters, Joseph

PATENT ASSIGNEE(S): Genetics Institute, Inc., USA

SOURCE: PCT Int. Appl., 47 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

INT. PATENT CLASSIF.:

MAIN: C12N015-27

CLASSIFICATION: 3-4 (Biochemical Genetics)

Section cross-reference(s): 9

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:



PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9012874	A2	19901101	WO 1990-US2144	19900419
WO 9012874	A3	19910110		
W: AU, CA, JP				
RW: AT, BE, CH, DE, DK, ES, FR, GB, IT, LU, NL, SE				
US 5166322	A	19921124	US 1989-341990	19890421
AU 9055537	A1	19901116	AU 1990-55537	19900419
EP 469074	A1	19920205	EP 1990-907849	19900419
EP 469074	B1	19960731		
R: AT, BE, CH, DE, DK, ES, FR, GB, IT, LI, LU, NL, SE				
JP 04504801	T2	19920827	JP 1990-507086	19900419
JP 2557144	B2	19961127		
EP 668353	A1	19950823	EP 1995-103989	19900419
R: AT, BE, CH, DE, DK, ES, FR, GB, IT, LI, LU, NL, SE				
EP 668354	A1	19950823	EP 1995-103990	19900419
R: AT, BE, CH, DE, DK, ES, FR, GB, IT, LI, LU, NL, SE				
AT 140969	E	19960815	AT 1990-907849	19900419
ES 2090132	T3	19961016	ES 1990-907849	19900419
PRIORITY APPLN. INFO.:			US 1989-341990	A 19890421
			EP 1990-907849	A3 19900419
			WO 1990-US2144	A 19900419

PATENT CLASSIFICATION CODES:

PATENT NO.	CLASS	PATENT FAMILY CLASSIFICATION CODES
WO 9012874	ICM	C12N015-27
	IPCI	C12N0015-27 [ICM,5]; C12N0015-19 [ICM,5,C*]
	IPCR	A61K0038-00 [I,C*]; A61K0038-00 [I,A]; A61K0047-48 [I,C*]; A61K0047-48 [I,A]; A61P0007-00 [I,C*]; A61P0007-00 [I,A]; A61P0007-06 [I,A]; C07K0014-00 [I,C*]; C07K0014-00 [I,A]; C07K0014-435 [I,C*]; C07K0014-505 [I,A]; C07K0014-52 [I,A]; C07K0014-53 [I,A]; C07K0014-535 [I,A]; C07K0014-54 [I,A]; C12N0001-21 [I,C*]; C12N0001-21 [I,A]; C12N0015-09 [I,C*]; C12N0015-09 [I,A]; C12N0015-19 [I,C*]; C12N0015-24 [I,A]; C12N0015-27 [I,A]; C12P0021-02 [I,C*]; C12P0021-02 [I,A]; C12R0001-19 [N,A]
US 5166322	IPCI	C07K0013-00 [ICM,5]; C12N0015-24 [ICS,5]; C12N0015-19 [ICS,5,C*]
	IPCR	A61K0038-00 [I,C*]; A61K0038-00 [I,A]; A61K0047-48 [I,C*]; A61K0047-48 [I,A]; A61P0007-00 [I,C*]; A61P0007-00 [I,A]; A61P0007-06 [I,A]; C07K0014-00 [I,C*]; C07K0014-00 [I,A]; C07K0014-435 [I,C*]; C07K0014-505 [I,A]; C07K0014-52 [I,A]; C07K0014-53 [I,A]; C07K0014-535 [I,A]; C07K0014-54 [I,A]; C12N0001-21 [I,C*]; C12N0001-21 [I,A]; C12N0015-09 [I,C*]; C12N0015-09 [I,A]; C12N0015-19 [I,C*]; C12N0015-24 [I,A]; C12N0015-27 [I,A]; C12P0021-02 [I,C*]; C12P0021-02 [I,A]; C12R0001-19 [N,A]
	NCL	530/351.000; 424/085.100; 424/085.200; 424/178.100; 435/069.500; 435/069.520; 525/054.100; 530/402.000; 530/403.000; 530/404.000; 530/405.000; 536/023.500; 536/023.510; 930/141.000
AU 9055537	IPCI	C12N0015-27 [ICM,5]; C12N0015-19 [ICM,5,C*]
	IPCR	A61K0038-00 [I,C*]; A61K0038-00 [I,A]; A61K0047-48 [I,C*]; A61K0047-48 [I,A]; A61P0007-00 [I,C*]; A61P0007-00 [I,A]; A61P0007-06 [I,A]; C07K0014-00 [I,C*]; C07K0014-00 [I,A]; C07K0014-435 [I,C*]; C07K0014-505 [I,A]; C07K0014-52 [I,A]; C07K0014-53 [I,A]; C07K0014-535 [I,A]; C07K0014-54 [I,A]; C12N0001-21 [I,C*]; C12N0001-21 [I,A]; C12N0015-09 [I,C*]; C12N0015-09 [I,A]; C12N0015-19 [I,C*]; C12N0015-24 [I,A]; C12N0015-27 [I,A]; C12P0021-02 [I,C*]; C12P0021-02 [I,A]; C12R0001-19 [N,A]
EP 469074	IPCI	C12N0015-27 [ICM,5]; C12N0015-19 [ICM,5,C*]



	IPCR	A61K0038-00 [I,C*]; A61K0038-00 [I,A]; A61K0047-48 [I,C*]; A61K0047-48 [I,A]; A61P0007-00 [I,C*]; A61P0007-00 [I,A]; A61P0007-06 [I,A]; C07K0014-00 [I,C*]; C07K0014-00 [I,A]; C07K0014-435 [I,C*]; C07K0014-505 [I,A]; C07K0014-52 [I,A]; C07K0014-53 [I,A]; C07K0014-535 [I,A]; C07K0014-54 [I,A]; C12N0001-21 [I,C*]; C12N0001-21 [I,A]; C12N0015-09 [I,C*]; C12N0015-09 [I,A]; C12N0015-19 [I,C*]; C12N0015-24 [I,A]; C12N0015-27 [I,A]; C12P0021-02 [I,C*]; C12P0021-02 [I,A]; C12R0001-19 [N,A]
JP 04504801	IPCI	C12P0021-02 [ICM,5]; A61K0037-02 [ICS,5]; C07K0013-00 [ICS,5]; C12N0001-21 [ICS,5]; C12N0015-12 [ICS,5]; C12N0015-24 [ICS,5]; C12N0015-19 [ICS,5,C*]; C12P0021-02 [ICI,5]; C12R0001-19 [ICI,5]
EP 668353	IPCI	C12N0015-27 [ICM,6]; C12N0015-19 [ICM,6,C*]; C07K0014-505 [ICS,6]; C12P0021-02 [ICS,6]; C07K0014-535 [ICS,6]; C07K0014-435 [ICS,6,C*]
	ECLA	C07K014/505; C07K014/535; C07K014/54A
EP 668354	IPCI	C12N0015-27 [ICM,6]; C12N0015-19 [ICM,6,C*]; C12P0021-02 [ICS,6]; C07K0014-535 [ICS,6]; C07K0014-435 [ICS,6,C*]
	ECLA	C07K014/535
AT 140969	IPCI	C12N0015-24 [ICM,6]; C12N0015-19 [ICM,6,C*]
	IPCR	A61K0038-00 [I,C*]; A61K0038-00 [I,A]; A61K0047-48 [I,C*]; A61K0047-48 [I,A]; A61P0007-00 [I,C*]; A61P0007-00 [I,A]; A61P0007-06 [I,A]; C07K0014-00 [I,C*]; C07K0014-00 [I,A]; C07K0014-435 [I,C*]; C07K0014-505 [I,A]; C07K0014-52 [I,A]; C07K0014-53 [I,A]; C07K0014-535 [I,A]; C07K0014-54 [I,A]; C12N0001-21 [I,C*]; C12N0001-21 [I,A]; C12N0015-09 [I,C*]; C12N0015-09 [I,A]; C12N0015-19 [I,C*]; C12N0015-24 [I,A]; C12N0015-27 [I,A]; C12P0021-02 [I,C*]; C12P0021-02 [I,A]; C12R0001-19 [N,A]
ES 2090132	IPCI	C12N0015-24 [ICM,6]; C12N0015-19 [ICM,6,C*]
	IPCR	A61K0038-00 [I,C*]; A61K0038-00 [I,A]; A61K0047-48 [I,C*]; A61K0047-48 [I,A]; A61P0007-00 [I,C*]; A61P0007-00 [I,A]; A61P0007-06 [I,A]; C07K0014-00 [I,C*]; C07K0014-00 [I,A]; C07K0014-435 [I,C*]; C07K0014-505 [I,A]; C07K0014-52 [I,A]; C07K0014-53 [I,A]; C07K0014-535 [I,A]; C07K0014-54 [I,A]; C12N0001-21 [I,C*]; C12N0001-21 [I,A]; C12N0015-09 [I,C*]; C12N0015-09 [I,A]; C12N0015-19 [I,C*]; C12N0015-24 [I,A]; C12N0015-27 [I,A]; C12P0021-02 [I,C*]; C12P0021-02 [I,A]; C12R0001-19 [N,A]

# ABSTRACT:

Analogues of polypeptides in which cysteines are substituted for other amino acids or are inserted [cysteine-added variants (CAVs)] are prepared by expression of the gene in an heterologous host. CAVs of human interleukin-3 (IL-3), granulocyte-colony stimulating factor (G-CSF) and erythropoietin (EPO) are prepared to improve their therapeutic efficacy. The method comprises substitution with or insertion of >1 cysteine residues to the natural proteins and, preferably, deletion of certain N-terminal amino acids and modification of the new cysteine sites by coupling of the thiol. More than 15 analogues of human IL-3 with modified N-termini, e.g. deletion of Ala-1, and addnl. cysteine residues at positions 3, 6, 8, 10, 12, 100, etc. were prepared by conventional oligonucleotide-mediated site-specific mutations and expression of the genes in animal or microbial hosts. HPLC-purified CAVs of IL-3 were refolded by reacting with a PEG derivative e.g. S-pyridyl monomethoxy PEG 5000 or maleimido monomethoxy PEG 5000. Biol. activities of these CAVs of IL-3 were also observed

SUPPL. TERM: cysteine addn variant polypeptide recombinant;  
interleukin 3 cysteine addn recombinant; granulocyte colony stimulating factor cysteine; erythropoietin cysteine addn recombinant

INDEX TERM: Proteins, biological studies  
 ROLE: BMF (Bioindustrial manufacture); BIOL (Biological study); PREP (Preparation)  
 (cysteine-added analogs of,  
 recombinant manufacture and refolding of)

INDEX TERM: Bacteria  
 Escherichia coli  
 Yeast  
 (expression in, of genes for cysteine-  
 added protein analogs, therapeutic efficacy in  
 relation to)

INDEX TERM: Gene and Genetic element, animal  
 ROLE: BAC (Biological activity or effector, except adverse);  
 BPR (Biological process); BSU (Biological study,  
 unclassified); BIOL (Biological study); PROC (Process)  
 (for cysteine-added protein analogs,  
 cloning and expression of, therapeutic efficacy in  
 relation to)

INDEX TERM: Protein sequences  
 (of cysteine-added analogs of  
 erythropoietin, of human, complete)

INDEX TERM: Protein sequences  
 (of cysteine-added analogs of  
 granulocyte colony-stimulating factor, of human complete)

INDEX TERM: Protein sequences  
 (of cysteine-added analogs of  
 interleukin-3, of human, complete)

INDEX TERM: Molecular cloning  
 (of genes for cysteine-added analogs  
 of proteins, therapeutic efficacy in relation to)

INDEX TERM: Erythropoiesis  
 (stimulation of, recombinant manufacture of erythropoietins  
 analogs containing addnl. cysteine residues for)

INDEX TERM: Animal cell line  
 (CHO, expression in, of genes for cysteine-  
 added protein analogs, therapeutic efficacy in  
 relation to)

INDEX TERM: Deoxyribonucleic acid sequences  
 (colony-stimulating factor-specifying, cysteine  
 -added analogs of, of human, complete)

INDEX TERM: Deoxyribonucleic acid sequences  
 (erythropoietin-specifying, cysteine-  
 added analogs of, of human, complete)

INDEX TERM: Lymphokines and Cytokines  
 ROLE: BMF (Bioindustrial manufacture); BIOL (Biological  
 study); PREP (Preparation)  
 (interleukin 3, cysteine-added  
 analogs of, recombinant manufacture and refolding of).

INDEX TERM: Deoxyribonucleic acid sequences  
 (interleukin 3-specifying, cysteine-  
 added analogs of, of human, complete)

INDEX TERM: 134373-40-3 134373-41-4 134373-42-5 134373-43-6  
 134373-44-7 134373-45-8 134373-46-9 134373-48-1  
 134373-49-2 134373-50-5 134373-51-6 134373-52-7  
 134373-58-3  
 ROLE: PRP (Properties)  
 (amino acid sequence of and cloning and expression in  
 eukaryotes or prokaryotes of gene for)

INDEX TERM: 134373-32-3 134373-33-4 134373-34-5 134373-35-6  
 134373-47-0 134373-53-8 134373-54-9 134373-55-0  
 134373-56-1 134373-57-2  
 ROLE: PRP (Properties)  
 (amino acid sequence of and cloning and expression in  
 prokaryotes or eukaryotes of gene for)

INDEX TERM: 11096-26-7P, Erythropoietin 62683-29-8P, Granulocyte

colony-stimulating factor  
ROLE: BMF (Bioindustrial manufacture); BIOL (Biological study); PREP (Preparation)  
(cysteine-added analogs of,  
recombinant manufacture and refolding of)

INDEX TERM: 134376-32-2 134376-33-3 134376-34-4 134376-35-5  
134376-36-6 134376-37-7 134376-38-8 134376-39-9  
134376-40-2 134376-41-3 134376-42-4 134376-43-5  
134376-44-6 134376-45-7 134376-46-8 134376-47-9  
134376-48-0 134376-49-1 134376-50-4 134376-51-5  
ROLE: PRP (Properties)

(nucleotide sequence and cloning and expression in prokaryotes or eukaryotes of)  
INDEX TERM: 52-90-4P, Cysteine, biological studies  
ROLE: BMF (Bioindustrial manufacture); BIOL (Biological study); PREP (Preparation)  
(polypeptide containing addnl., recombinant manufacture and refolding of)

INDEX TERM: 25322-68-3DP, thiol group-reactive derivs. 68181-17-9DP,  
N-Succinimidyl-3-(2-pyridyldithio)propionate, reaction products with PEG 5000 133793-66-5P 133793-67-6P  
133864-89-8DP, reaction products with PEG 5000  
ROLE: SPN (Synthetic preparation); PREP (Preparation)  
(preparation and use of, in refolding of cysteine-added recombinant interleukin-3 and other polypeptides)

=> D Iall L10 1-2

L10 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2005:238537 CAPLUS  
DOCUMENT NUMBER: 142:310938  
ENTRY DATE: Entered STN: 18 Mar 2005  
TITLE: Fusion proteins composed of carotenoid-binding peptides and phenol oxidizing enzymes (phenol oxidase B or laccase), and their use in construction of detergent for bleaching carotenoid stain on fabric or surface  
INVENTOR(S): Wolfgang, Aehle; Baldwin, Toby M.; Van Gastel, Franciscus J. C.; Janssen, Giselle G.; Murray, Christopher J.; Wang, Huaming; Winetzky, Deborah S.  
PATENT ASSIGNEE(S): Neth.  
SOURCE: U.S. Pat. Appl. Publ., 107 pp.  
CODEN: USXXCO  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
INT. PATENT CLASSIF.:  
MAIN: C12Q001-68  
SECONDARY: C07H021-04; C12N009-02; C11D003-386  
US PATENT CLASSIF.: 435006000; 435069100; 435189000; 435320100; 435325000; 536023200; 510320000  
CLASSIFICATION: 3-2 (Biochemical Genetics)  
Section cross-reference(s): 7, 10, 46  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2005058996	A1	20050317	US 2002-235043	20020903
PRIORITY APPLN. INFO.:			US 2002-235043	20020903

PATENT CLASSIFICATION CODES:

PATENT NO.	CLASS	PATENT FAMILY CLASSIFICATION CODES
US 2005058996	ICM	C12Q001-68

ICS C07H021-04; C12N009-02; C11D003-386  
 INCL 435006000; 435069100; 435189000; 435320100; 435325000;  
 536023200; 510320000  
 IPCI C12Q0001-68 [ICM,7]; C07H0021-04 [ICS,7]; C07H0021-00  
 [ICS,7,C\*]; C12N0009-02 [ICS,7]; C11D0003-386 [ICS,7];  
 C11D0003-38 [ICS,7,C\*]  
 IPCR C11D0003-38 [I,C\*]; C11D0003-386 [I,A]; C12N0009-02  
 [I,C\*]; C12N0009-02 [I,A]  
 NCL 435/006.000; 435/069.100; 435/189.000; 435/320.100;  
 435/325.000; 510/320.000; 536/023.200  
 ECLA C11D003/386H; C12N009/02H3B2

ABSTRACT:

The invention provides numerous peptides composed of 7 or 12 amino acids that are able to bind to a carotenoid compound on a fabric. The invention relates said peptides: (a) may also contain a cysteine added to each end (defined as C-C derivs.); (b) comprise a repeatable motif; and (c) bind to tomato or paprika stains on cotton. The invention also provides chimeric proteins composed of said peptides linked to the C-  
 \*\*\*terminus\*\*\* of a phenol oxidizing enzyme, such as laccase and/or Stachybotrys phenol oxidase B, and/or variants thereof. The invention further provides polynucleotides encoding said carotenoid-binding peptide-phenol oxidizing enzyme fusion proteins, and use of said polynucleotides in transforming host cells for recombinant production of fusion proteins. Still further, the invention provides for the use of said recombinant fusion proteins in construction of a detergent, wherein said detergent can be used to clean a carotenoid stain on a fabric and/or a surface contacting the stain. Finally, the invention provides the amino acid sequences of Stachybotrys chartarum phenol oxidase B and carotenoid-binding peptides. In the examples, the invention demonstrated that a fusion protein composed of Seq ID number 16 carotenoid-binding peptide and a phenol oxidase B variant (M254F/E346V/E348Q) bound to tomato stain on cotton better than the enzyme alone.

SUPPL. TERM: fusion protein carotenoid binding peptide phenol oxidizing enzyme; sequence phenol oxidase B Stachybotrys use fusion protein; detergent stain remover carotenoid binding peptide phenol oxidizing enzyme; laccase carotenoid binding peptide fusion detergent stain remover

INDEX TERM: Detergents  
 (bleaching; fusion proteins composed of carotenoid-binding peptides and phenol oxidizing enzymes, and their use in construction of detergent for bleaching carotenoid stain on fabric or surface)

INDEX TERM: Fusion proteins (chimeric proteins)  
 ROLE: BPN (Biosynthetic preparation); PRP (Properties); TEM (Technical or engineered material use); BIOL (Biological study); PREP (Preparation); USES (Uses)  
 (carotenoid-binding peptide fused to phenol oxidizing enzyme; fusion proteins composed of carotenoid-binding peptides and phenol oxidizing enzymes, their polynucleotides and use in construction of detergent)

INDEX TERM: Peptides, properties  
 ROLE: BPN (Biosynthetic preparation); PRP (Properties); TEM (Technical or engineered material use); BIOL (Biological study); PREP (Preparation); USES (Uses)  
 (carotenoid-binding, fusion product with phenol oxidizing enzyme; fusion proteins composed of carotenoid-binding peptides and phenol oxidizing enzymes, and their use in construction of detergent)

INDEX TERM: Textiles  
 (cotton; fusion proteins composed of carotenoid-binding peptides and phenol oxidizing enzymes, and their ability to better bind tomato stains on cotton than enzymes alone)

INDEX TERM: Detergents

Surface  
Textiles

(fusion proteins composed of carotenoid-binding peptides and phenol oxidizing enzymes, and their use in construction of detergent for bleaching carotenoid stain on fabric or surface)

INDEX TERM:

Carotenes, processes

ROLE: REM (Removal or disposal); PROC (Process)

(fusion proteins composed of carotenoid-binding peptides and phenol oxidizing enzymes, and their use in construction of detergent for bleaching carotenoid stain on fabric or surface)

INDEX TERM:

Polynucleotides

ROLE: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)

(fusion proteins composed of carotenoid-binding peptides and phenol oxidizing enzymes, their polynucleotides and use in construction of detergent for bleaching carotenoid stain on fabric or surface)

INDEX TERM:

Capsicum annuum annuum

(longum group, paprika; peptides able to bind carotenoid compds., their ability to bind carotenoids found in tomato or paprika and use in construction of fusion proteins with Stachybotrys phenol oxidizing enzymes)

INDEX TERM:

Plasmid vectors

(pGAPT; polynucleotides encoding fusion proteins composed of carotenoid-binding peptides and phenol oxidizing enzymes, their vectors and use in transforming host cells for recombinant protein production)

INDEX TERM:

Lycopersicon esculentum

(peptides able to bind carotenoid compds., their ability to bind carotenoids found in tomato or paprika and use in construction of fusion proteins with Stachybotrys phenol oxidizing enzymes)

INDEX TERM:

Repeat motifs (protein)

(peptides able to bind carotenoid compds., their protein motifs, sequences and use in construction of fusion proteins with Stachybotrys phenol oxidizing enzymes for creation of detergent)

INDEX TERM:

Molecular cloning

(polynucleotides encoding fusion proteins composed of carotenoid-binding peptides and phenol oxidizing enzymes, their vectors and use in transforming host cells for recombinant protein production)

INDEX TERM:

Stachybotrys chartarum

(sequence of phenol oxidase B found in Stachybotrys chartarum, and its use in production of fusion proteins comprising carotenoid-binding proteins)

INDEX TERM:

Detergents

(stain removers; fusion proteins composed of carotenoid-binding peptides and phenol oxidizing enzymes, and their use in construction of detergent for bleaching carotenoid stain on fabric or surface)

INDEX TERM:

Aspergillus niger

Escherichia coli

(transformed; polynucleotides encoding fusion proteins composed of carotenoid-binding peptides and phenol oxidizing enzymes, their vectors and use in transforming host cells for recombinant protein production)

INDEX TERM:

847966-56-7P

ROLE: BPN (Biosynthetic preparation); PRP (Properties); TEM (Technical or engineered material use); BIOL (Biological study); PREP (Preparation); USES (Uses)

(amino acid sequence; fusion proteins composed of carotenoid-binding peptides and phenol oxidizing enzymes,



and fusion product with phenol oxidizing enzyme  
847910-77-4DP, cysteine derivs. and fusion product with  
phenol oxidizing enzyme 847910-78-5DP, cysteine derivs.  
and fusion product with phenol oxidizing enzyme  
847910-84-3DP, cysteine derivs. and fusion product with  
phenol oxidizing enzyme

ROLE: BPN (Biosynthetic preparation); PRP (Properties); TEM  
(Technical or engineered material use); BIOL (Biological  
study); PREP (Preparation); USES (Uses)

(amino acid sequence; peptides able to bind carotenoid  
compds., their sequences and use in construction of  
fusion proteins with Stachybotrys phenol oxidizing  
enzymes for creation of detergent)

INDEX TERM: 847966-55-6DP, fusion product with carotenoid-binding  
peptide

ROLE: BPN (Biosynthetic preparation); PRP (Properties); TEM  
(Technical or engineered material use); BIOL (Biological  
study); PREP (Preparation); USES (Uses)

(amino acid sequence; sequence of phenol oxidase B found  
in Stachybotrys chartarum, and its use in production of  
fusion proteins comprising carotenoid-binding proteins)

INDEX TERM: 9002-10-2DP, Phenol oxidase, fusion product with  
carotenoid-binding peptide

ROLE: BPN (Biosynthetic preparation); PRP (Properties); TEM  
(Technical or engineered material use); BIOL (Biological  
study); PREP (Preparation); USES (Uses)

(isoenzyme B; peptides able to bind carotenoid compds.,  
their sequences and use in construction of fusion  
proteins with Stachybotrys phenol oxidizing enzymes (such  
as phenol oxidase B) for creation of detergent)

INDEX TERM: 80498-15-3DP, Laccase, fusion product with  
carotenoid-binding peptide

ROLE: BPN (Biosynthetic preparation); PRP (Properties); TEM  
(Technical or engineered material use); BIOL (Biological  
study); PREP (Preparation); USES (Uses)

(peptides able to bind carotenoid compds., their  
sequences and use in construction of fusion proteins with  
Stachybotrys phenol oxidizing enzymes (such as laccase)  
for creation of detergent)

INDEX TERM: 52-90-4DP, Cysteine, added to N-terminus  
or C-terminus of carotenoid-binding  
peptide

ROLE: BPN (Biosynthetic preparation); PRP (Properties); TEM  
(Technical or engineered material use); BIOL (Biological  
study); PREP (Preparation); USES (Uses)

(peptides able to bind carotenoid compds., their  
sequences and use in construction of fusion proteins with  
Stachybotrys phenol oxidizing enzymes for creation of  
detergent)

INDEX TERM: 502697-64-5 502697-65-6 847967-48-0 847967-49-1  
847967-50-4 847967-51-5 847967-52-6 847967-53-7  
847967-54-8 847967-55-9 847967-56-0 847967-57-1  
847967-58-2

ROLE: PRP (Properties)

(unclaimed sequence; fusion proteins composed of  
carotenoid-binding peptides and phenol oxidizing enzymes  
(phenol oxidase B or laccase), and their use in  
construction of detergent for bleaching carotenoid stain  
on fabric or surface)

L10 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2004:601396 CAPLUS

DOCUMENT NUMBER: 141:273856

ENTRY DATE: Entered STN: 28 Jul 2004

TITLE: Synthesis of fluorescent oligonucleotide-EYFP



conjugate: Towards supramolecular construction of semisynthetic biomolecular antennae

AUTHOR(S): Kukolka, Florian; Niemeyer, Christof M.

CORPORATE SOURCE: Fachbereich Chemie Biologisch-Chemische Mikrostrukturtechnik, Universitaet Dortmund, Dortmund, D-44227, Germany

SOURCE: Organic & Biomolecular Chemistry (2004), 2(15), 2203-2206  
CODEN: OBCRAK; ISSN: 1477-0520

PUBLISHER: Royal Society of Chemistry

DOCUMENT TYPE: Journal

LANGUAGE: English

CLASSIFICATION: 9-14 (Biochemical Methods)

ABSTRACT:  
A novel species of DNA-protein conjugate was synthesized by chemical linking DNA oligonucleotides to Aequorea victoria green fluorescent protein mutant EYFP. An addnl. cysteine was added to the C-  
\*\*\*terminus\*\*\* of the EYFP by genetic engineering and used to covalently attach amino-modified oligonucleotide with the aid of the heterobifunctional crosslinker sSMCC. EYFP maintained its fluorescence upon conjugation. The oligonucleotide provides an addnl. binding site to the fluorescent protein, and hence, the EYFP conjugate could be specifically hybridized with both complementary DNA-protein conjugates in-solution as well as immobilized at capture oligonucleotides attached to a solid substrate. These studies are paving the way for future applications in the self-assembly of photoactive supramol. complexes, such as artificial light-harvesting systems.

SUPPL. TERM: synthesis fluorescence oligonucleotide EYFP conjugate

INDEX TERM: DNA  
ROLE: ARU (Analytical role, unclassified); PEP (Physical, engineering or chemical process); PYP (Physical process); ANST (Analytical study); PROC (Process)  
(conjugates; synthesis of fluorescent oligonucleotide-EYFP conjugate)

INDEX TERM: Proteins  
ROLE: BSU (Biological study, unclassified); BIOL (Biological study)  
(conjugates; synthesis of fluorescent oligonucleotide-EYFP conjugate)

INDEX TERM: Proteins  
ROLE: BSU (Biological study, unclassified); BIOL (Biological study)  
(green fluorescent; synthesis of fluorescent oligonucleotide-EYFP conjugate)

INDEX TERM: Aequorea victoria  
Nucleic acid hybridization  
Self-assembly  
Supramolecular structure  
(synthesis of fluorescent oligonucleotide-EYFP conjugate)

INDEX TERM: 7647-14-5, Sodium chloride, analysis  
ROLE: ARU (Analytical role, unclassified); ANST (Analytical study)  
(synthesis of fluorescent oligonucleotide-EYFP conjugate)

INDEX TERM: 64987-85-5D, SMCC, DNA conjugates  
ROLE: ARU (Analytical role, unclassified); PEP (Physical, engineering or chemical process); PYP (Physical process); ANST (Analytical study); PROC (Process)  
(synthesis of fluorescent oligonucleotide-EYFP conjugate)

REFERENCE COUNT: 22 THERE ARE 22 CITED REFERENCES AVAILABLE FOR THIS RECORD.

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L11 ANSWER 1 OF 5 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2005:1355291 CAPLUS

DOCUMENT NUMBER: 144:47224

ENTRY DATE: Entered STN: 30 Dec 2005

TITLE: Heat stability of maize endosperm ADP-glucose pyrophosphorylase is enhanced by insertion of a cysteine in the N terminus of the small subunit

AUTHOR(S): Linebarger, Carla R. Lyerly; Boehlein, Susan K.; Sewell, Aileen K.; Shaw, Janine; Hannah, L. Curtis  
 CORPORATE SOURCE: Program in Plant Molecular and Cellular Biology and Horticultural Sciences, University of Florida, Gainesville, FL, 32610-0245, USA

SOURCE: Plant Physiology (2005), 139(4), 1625-1634  
 CODEN: PLPHAY; ISSN: 0032-0889

PUBLISHER: American Society of Plant Biologists

DOCUMENT TYPE: Journal

LANGUAGE: English

CLASSIFICATION: 7-5 (Enzymes)

ABSTRACT:

ADP-glucose pyrophosphorylase (AGPase) is a key regulatory enzyme in starch biosynthesis. However, plant AGPases differ in several parameters, including spatial and temporal expression, allosteric regulation, and thermostability. AGPases of cereal endosperms are thermolabile, whereas those in other tissues, such as the potato tuber, are thermostable. Sequence comparisons of thermostable and thermolabile AGPases identified an N-terminal motif unique to the thermostable enzymes. Insertion of this motif into recombinant maize endosperm AGPase increased the half-life at 58° by >70-fold. The Km values for physiol. substrates were unaffected, although the kcat was doubled. A Cys residue within the inserted motif gave rise to small subunit homodimers not found in the wild-type maize enzyme. Placement of this N-terminal motif into a mosaic small subunit containing the N-terminus from maize endosperm and the C-terminus from potato tuber AGPase increased the thermostability by >300-fold.

SUPPL. TERM: ADPglucose pyrophosphorylase maize thermostability enhancement cysteine insertion protein engineering

INDEX TERM: Seed  
(endosperm; enhancement of thermostability of maize endosperm ADP-glucose pyrophosphorylase by insertion of Cys residue into small subunit N-terminal region)

INDEX TERM: Thermal stability  
Zea mays  
(enhancement of thermostability of maize endosperm ADP-glucose pyrophosphorylase by insertion of Cys residue into small subunit N-terminal region)

INDEX TERM: Enzyme kinetics  
Michaelis constant  
(of ADP-glucose pyrophosphorylase wild-type and mutant forms of maize endosperm)

INDEX TERM: 52-90-4, L-Cysteine, biological studies  
ROLE: BSU (Biological study, unclassified); BIOL (Biological study)  
(enhancement of thermostability of maize endosperm ADP-glucose pyrophosphorylase by insertion of Cys residue into small subunit N-terminal region)

INDEX TERM: 9027-71-8, ADP-glucose pyrophosphorylase  
ROLE: BSU (Biological study, unclassified); PEP (Physical, engineering or chemical process); PRP (Properties); PYP (Physical process); BIOL (Biological study); PROC (Process)  
(small subunit, mutants; enhancement of thermostability of maize endosperm ADP-glucose pyrophosphorylase by insertion of Cys residue into small subunit N-terminal region)

REFERENCE COUNT: 33 THERE ARE 33 CITED REFERENCES AVAILABLE FOR THIS RECORD.

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L11 ANSWER 2 OF 5 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2003:892083 CAPLUS  
 DOCUMENT NUMBER: 139:376203  
 ENTRY DATE: Entered STN: 14 Nov 2003  
 TITLE: Construction of recombinant calcium-binding  
 cysteine-inserted photoproteins and  
 their conjugates with ligands for analytical use  
 INVENTOR(S): Inouye, Satoshi  
 PATENT ASSIGNEE(S): Chisso Corporation, Japan  
 SOURCE: U.S. Pat. Appl. Publ., 15 pp.  
 CODEN: USXXCO  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 INT. PATENT CLASSIF.:  
 MAIN: G01N033-53  
 SECONDARY: C07H021-04; C07K014-435; C12P021-02; C12N005-06  
 US PATENT CLASSIF.: 530350000; 435007500; 435069100; 435320100; 435325000;  
 536023500  
 CLASSIFICATION: 3-2 (Biochemical Genetics)  
 Section cross-reference(s): 9  
 FAMILY ACC. NUM. COUNT: 1  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003212259	A1	20031113	US 2003-400630	20030328
JP 2004000143	A2	20040108	JP 2003-17505	20030127
PRIORITY APPLN. INFO.:			JP 2002-93849	A 20020329
			JP 2002-17505	A 20030127

PATENT CLASSIFICATION CODES:

PATENT NO.	CLASS	PATENT FAMILY CLASSIFICATION CODES
US 2003212259	ICM	G01N033-53
	ICS	C07H021-04; C07K014-435; C12P021-02; C12N005-06
	INCL	530350000; 435007500; 435069100; 435320100; 435325000; 536023500
	IPCI	G01N0033-53 [ICM,7]; C07H0021-04 [ICS,7]; C07H0021-00 [ICS,7,C*]; C07K0014-435 [ICS,7]; C12P0021-02 [ICS,7]; C12N0005-06 [ICS,7]
	IPCR	G01N0033-53 [I,C*]; G01N0033-53 [I,A]; C07K0014-435 [I,C*]; C07K0014-435 [I,A]; C12N0001-15 [I,C*]; C12N0001-15 [I,A]; C12N0001-19 [I,C*]; C12N0001-19 [I,A]; C12N0001-21 [I,C*]; C12N0001-21 [I,A]; C12N0005-10 [I,C*]; C12N0005-10 [I,A]; C12N0015-09 [I,C*]; C12N0015-09 [I,A]; C12P0021-02 [I,C*]; C12P0021-02 [I,A]; G01N0033-531 [I,C*]; G01N0033-531 [I,A]
	NCL	530/350.000; 435/007.500; 435/069.100; 435/320.100; 435/325.000; 536/023.500
	ECLA	C07K014/435A5
JP 2004000143	IPCI	C12N0015-09 [ICM,7]; C07K0014-435 [ICS,7]; C12N0001-15 [ICS,7]; C12N0001-19 [ICS,7]; C12N0001-21 [ICS,7]; C12N0005-10 [ICS,7]; C12P0021-02 [ICS,7]; G01N0033-53 [ICS,7]; G01N0033-531 [ICS,7]; C12N0005-00 [ICS,7]
	IPCR	G01N0033-53 [I,C*]; G01N0033-53 [I,A]; C07K0014-435 [I,C*]; C07K0014-435 [I,A]; C12N0001-15 [I,C*]; C12N0001-15 [I,A]; C12N0001-19 [I,C*]; C12N0001-19 [I,A]; C12N0001-21 [I,C*]; C12N0001-21 [I,A];

C12N0005-10 [I,C\*]; C12N0005-10 [I,A]; C12N0015-09 [I,C\*]; C12N0015-09 [I,A]; C12P0021-02 [I,C\*]; C12P0021-02 [I,A]; G01N0033-531 [I,C\*]; G01N0033-531 [I,A]

FTERM 4B024/AA11; 4B024/BA80; 4B024/CA02; 4B024/EA04;  
 4B064/AG01; 4B064/CA19; 4B064/CC24; 4B064/DA13;  
 4B065/AB01; 4B065/BA02; 4B065/CA24; 4B065/CA46;  
 4H045/AA10; 4H045/AA20; 4H045/AA30; 4H045/BA10;  
 4H045/BA70; 4H045/CA50; 4H045/EA50; 4H045/FA74

ABSTRACT:

This invention is to provide a photoprotein which binds with a ligand specific for a substance to be detected at a binding ratio of 1:1 such that the luminescence activity is not reduced by binding with the ligand, a conjugate comprising the luminescent photoprotein and ligand, and a substance detection method which employs the conjugate as a marker. A calcium-binding photoprotein (aequorin) is produced having cysteine residue introduced within the 4th amino acid residue from the amino-terminus. A conjugate is formed by binding a ligand specific for a substance (biotin) to be detected to the calcium-binding photoprotein, in a binding ratio of 1:1, via the introduced cysteine residue. The conjugate may be utilized as a marker for a substance to be detected. Exemplary construction, culturing, purification, and biotinylation of \*\*\*cysteine\*\*\* -inserted aequorin is described.

SUPPL. TERM: cysteine inserted recombinant calcium  
 binding photoprotein conjugate marker; biotinylation  
 recombinant cysteine inserted aequorin  
 marker

INDEX TERM: Aequorea aequorea  
 (aequorin from; construction of recombinant  
 calcium-binding cysteine-inserted  
 photoproteins and their conjugates with ligands for anal.  
 use)

INDEX TERM: Proteins  
 ROLE: ARG (Analytical reagent use); BPN (Biosynthetic  
 preparation); PRP (Properties); ANST (Analytical study);  
 BIOL (Biological study); PREP (Preparation); USES (Uses)  
 (berovins; construction of recombinant calcium-binding  
 cysteine-inserted photoproteins and  
 their conjugates with ligands for anal. use)

INDEX TERM: Proteins  
 ROLE: ARG (Analytical reagent use); BPN (Biosynthetic  
 preparation); PRP (Properties); ANST (Analytical study);  
 BIOL (Biological study); PREP (Preparation); USES (Uses)  
 (clytins; construction of recombinant calcium-binding  
 cysteine-inserted photoproteins and  
 their conjugates with ligands for anal. use)

INDEX TERM: Ligands  
 ROLE: ARG (Analytical reagent use); SPN (Synthetic  
 preparation); ANST (Analytical study); PREP (Preparation);  
 USES (Uses)  
 (conjugated; construction of recombinant calcium-binding  
 cysteine-inserted photoproteins and  
 their conjugates with ligands for anal. use)

INDEX TERM: Antibodies and Immunoglobulins  
 Antigens  
 Avidins  
 Enzymes, preparation  
 Nucleic acids  
 Polysaccharides, preparation  
 Receptors  
 ROLE: ARG (Analytical reagent use); SPN (Synthetic  
 preparation); ANST (Analytical study); PREP (Preparation);  
 USES (Uses)  
 (conjugates; construction of recombinant calcium-binding  
 cysteine-inserted photoproteins and



L11 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2003:624906 CAPLUS

DOCUMENT NUMBER: 140:187111

ENTRY DATE: Entered STN: 14 Aug 2003

TITLE: Monitoring manufacturing process yields, purity and stability of structural variants of PEGylated staphylokinase mutant SY161 by quantitative reverse-phase chromatography

AUTHOR(S): Johnson, Catharine; Royal, Mabel; Moreadith, Randall; Bedu-Addo, Frank; Advant, Siddharth; Wan, Min; Conn, Greg

CORPORATE SOURCE: Diosynth-RTP, Pharmaceutical Sciences, Diosynth-RTP, Cary, NC, 27513, USA

SOURCE: Biomedical Chromatography (2003), 17(5), 335-344

CODEN: BICHE2; ISSN: 0269-3879

PUBLISHER: John Wiley & Sons Ltd.

DOCUMENT TYPE: Journal

LANGUAGE: English

CLASSIFICATION: 63-5 (Pharmaceuticals)

ABSTRACT:

Staphylokinase variant SY161 is a recombinant mutant of the Staphylococcus aureus polypeptide staphylokinase (Sak), and is currently in human clin. trials as a thrombolytic agent. The 15 kDa single chain SY161 protein is expressed as a soluble cytoplasmic product in E. coli with a single \*\*\*cysteine\*\*\* inserted near the N-terminus.

The protein as extracted from E. coli is a mixture of both monomeric and intermolecularly disulfide crosslinked species. To improve protein purification yields SY161 is sulfitolyzed during the early stages of production, preventing disulfide formation. The protein is later modified during manufacturing to incorporate a single 5 kDa polyethylene glycol group on the single sulfhydryl sidechain. We have developed and qualified a reverse-phase chromatog. method to quantitate SY161 during product manufacturing. We discuss the use of the assay during manufacturing development to monitor fermentation yields, the SY161 PEGylation

reaction, and as an in-process manufacturing control assay. The assay has been applied as a product purity and identity release assay and is suitable for use in assessing product structural integrity during stability testing. The assay has a linear range of quantitation for SY161 from at least 0.15 to 16 µg, and is in addition capable of detecting and quantitating protein de-PEGylation events and host cell-derived protein contaminants.

SUPPL. TERM: staphylokinase SY161 polyethylene glycol

INDEX TERM: Escherichia coli

Reversed phase chromatography

Stability

(monitoring manufacturing process yields, purity and stability of structural variants of PEGylated staphylokinase mutant SY161 by quant. reverse-phase chromatog.)

INDEX TERM: Polyoxyalkylenes, biological studies

ROLE: PRP (Properties); SPN (Synthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)

(reaction product with Staphylokinase SY161 mutant; monitoring manufacturing process yields, purity and stability of structural variants of PEGylated staphylokinase mutant SY161 by quant. reverse-phase chromatog.)

INDEX TERM: 9040-61-3DP, Staphylokinase, reaction product with PEG

ROLE: PRP (Properties); SPN (Synthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)

(SY161 mutant; monitoring manufacturing process yields, purity and stability of structural



variants of PEGylated staphylokinase  
mutant SY161 by quant. reverse-phase chromatog.)

INDEX TERM: 25322-68-3DP, Polyethylene glycol, reaction product with  
Staphylokinase SY161 mutant  
ROLE: PRP (Properties); SPN (Synthetic preparation); THU  
(Therapeutic use); BIOL (Biological study); PREP  
(Preparation); USES (Uses)  
(monitoring manufacturing process yields, purity and stability  
of structural variants of PEGylated  
staphylokinase mutant SY161 by quant.  
reverse-phase chromatog.)

REFERENCE COUNT: 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS  
RECORD.

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L11 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2001:491013 CAPLUS  
DOCUMENT NUMBER: 135:208715  
ENTRY DATE: Entered STN: 08 Jul 2001  
TITLE: Ca<sup>2+</sup>- and cross-bridge-dependent changes in N- and C-terminal structure of troponin C in rat cardiac muscle  
AUTHOR(S): Martyn, Donald A.; Regnier, Michael; Xu, Daguang; Gordon, Albert M.  
CORPORATE SOURCE: Department of Bioengineering, University of Washington, Seattle, WA, 98195, USA  
SOURCE: Biophysical Journal (2001), 80(1), 360-370  
CODEN: BIOJAU; ISSN: 0006-3495  
PUBLISHER: Biophysical Society  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
CLASSIFICATION: 13-6 (Mammalian Biochemistry)  
ABSTRACT:  
Linear dichroism of 5'-tetramethylrhodamine (5'ATR)-labeled cardiac troponin C (cTnC) was measured to monitor cTnC structure during Ca<sup>2+</sup>-activation of force in rat skinned myocardium. Mono-cysteine mutants allowed labeling at Cys-84 (cTnC(C84), near the D/E helix linker); Cys-35 (cTnC(C35), at nonfunctional site I); or near the C-terminus with a \*\*\*cysteine\*\*\* inserted at site 98 (cTnC-C35S, C84S, S98C, cTnC(C98)). With 5'ATR-labeled cTnC(C84) and cTnC(C98) dichroism increased with increasing [Ca<sup>2+</sup>], while rigor cross-bridges caused dichroism to increase more with 5'ATR-labeled cTnC(C84) than cTnC(C98). The pCa<sub>50</sub> values and nH from Hill anal. of the Ca<sup>2+</sup>-dependence of force and dichroism were 6.4 (±0.02) and 1.08 (±0.04) for force and 6.3 (±0.04) and 1.02 (±0.09) (n = 5) for dichroism in cTnC(C84) reconstituted trabeculae. Corresponding data from cTnC(C98) reconstituted trabeculae were 5.53 (±0.03) and 3.1 (±0.17) for force, and 5.39 (±0.03) and 1.87 (±0.17) (n = 5) for dichroism. The contribution of active cycling cross-bridges to changes in cTnC structure was determined by inhibition of force to 6% of pCa 4.0 controls with 1.0 mM sodium vanadate (Vi). With 5'ATR-labeled cTnC(C84) Vi caused both the pCa<sub>50</sub> of dichroism and the maximum value at pCa 4.0 to decrease, while with 5'ATR-labeled cTnC(C98) the pCa<sub>50</sub> of dichroism decreased with no change of dichroism at pCa 4.0. The dichroism of 5'ATR-labeled cTnC(C35) was insensitive to either Ca<sup>2+</sup> or strong cross-bridges. These data suggest that both Ca<sup>2+</sup> and cycling cross-bridges perturb the N-terminal structure of cTnC at Cys-84, while C-terminal structure is altered by site II Ca<sup>2+</sup>-binding, but not cross-bridges.  
SUPPL. TERM: troponin C calcium myosin crossbridge heart contraction  
INDEX TERM: Troponins  
ROLE: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)  
(C; Ca<sup>2+</sup>- and myosin cross-bridge-dependent changes in N- and C-terminal structure of troponin C in rat cardiac muscle)  
INDEX TERM: Cardiac contraction  
Dichroism  
(Ca<sup>2+</sup>- and myosin cross-bridge-dependent changes in N- and C-terminal structure of troponin C in rat cardiac muscle)  
INDEX TERM: Myosins  
ROLE: BPR (Biological process); BSU (Biological study,

unclassified); BIOL (Biological study); PROC (Process)  
(Ca<sup>2+</sup>- and myosin cross-bridge-dependent changes in N- and C-terminal structure of troponin C in rat cardiac muscle)

INDEX TERM: Microfilament  
(thin filament; Ca<sup>2+</sup>- and myosin cross-bridge-dependent changes in N- and C-terminal structure of troponin C in rat cardiac muscle)

INDEX TERM: Heart  
(trabecula; Ca<sup>2+</sup>- and myosin cross-bridge-dependent changes in N- and C-terminal structure of troponin C in rat cardiac muscle)

INDEX TERM: 7440-70-2, Calcium, biological studies  
ROLE: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)  
(Ca<sup>2+</sup>- and myosin cross-bridge-dependent changes in N- and C-terminal structure of troponin C in rat cardiac muscle)

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L11 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1992:402910 CAPLUS

DOCUMENT NUMBER: 117:2910

ENTRY DATE: Entered STN: 11 Jul 1992

TITLE: The anticodon and discriminator base are major determinants of cysteine tRNA identity in vivo

AUTHOR(S): Pallanck, Leo; Li, Shihong; Schulman, LaDonne H.

CORPORATE SOURCE: Dep. Dev. Biol. Cancer, Albert Einstein Coll. Med., Bronx, NY, 10461, USA

SOURCE: Journal of Biological Chemistry (1992), 267(11), 7221-3

CODEN: JBCHA3; ISSN: 0021-9258

DOCUMENT TYPE: Journal

LANGUAGE: English

CLASSIFICATION: 6-2 (General Biochemistry)

Section cross-reference(s): 7

# ABSTRACT:

Mutants of the Escherichia coli initiator tRNA (tRNA<sup>fMet</sup>) have been used to examine the role of the anticodon and discriminator base in in vivo aminoacylation of tRNAs by cysteinyl-tRNA synthetase. Substitution of the methionine anticodon CAU with the cysteine anticodon GCA was found to allow initiation of protein synthesis by the mutant tRNA from a complementary initiation codon in a reporter protein. Sequencing of the protein revealed that cysteine comprised about half of the amino acid at the \*\*\*N\*\*\* terminus. An addnl. mutation, converting the discriminator base of tRNA<sup>GCAfMet</sup> from A73 to the base present in tRNA<sup>Cys</sup>(U73), resulted in a 6-fold increase in the amount of protein produced and insertion of ≥90% cysteine in response to the complementary initiation codon. Substitution of C73 or G73 at the discriminator position led to insertion of little or no cysteine, indicating the importance of U73 for recognition of the tRNA by cysteinyl-tRNA synthetase. Single base changes in the anticodon of tRNA<sup>GCAfMet</sup> containing U73 from GCA to UCA, GUA, GCC, and GCG (changes underlined) eliminated or dramatically reduced cysteine insertion by the \*\*\*mutant\*\*\* initiator tRNA indicating that all three cysteine anticodon bases are essential for specific aminoacylation of the tRNA with cysteine in vivo.

SUPPL. TERM: cysteine tRNA aminoacylation anticodon discriminator base; cysteinyl tRNA synthetase tRNA recognition specificity

INDEX TERM: Escherichia coli  
(tRNA<sup>fMet</sup> mutant form, of cysteinyl-tRNA synthetase recognition of, anticodon and discriminator base dependence of)

INDEX TERM: Ribonucleic acids, transfer  
ROLE: BIOL (Biological study)  
(anticodons, of cysteine tRNA, aminoacylation specificity dependence on)

INDEX TERM: Ribonucleic acids, transfer  
ROLE: BIOL (Biological study)  
(cysteine-specific, cysteinyl-tRNA synthetase recognition of, anticodon and discriminator base dependence of)

INDEX TERM: Ribonucleic acids, transfer  
ROLE: BIOL (Biological study)  
(formylatable methionine-specific CAU, cysteinyl-tRNA synthetase recognition of mutant forms of, of Escherichia coli, anticodon and discriminator base dependence of)

INDEX TERM: 66-22-8, Uracil, biological studies  
ROLE: BIOL (Biological study)

(in tRNA<sup>f</sup>Met mutant, of Escherichia coli,  
adenine-73 replaced by, cysteinyl-tRNA synthetase  
recognition dependence on)

INDEX TERM: 73-24-5, Adenine, biological studies  
ROLE: BIOL (Biological study)  
(in tRNA<sup>f</sup>Met position 73 of Escherichia coli,  
aminoacylation specificity in relation to)

INDEX TERM: 37318-56-2, Cysteinyl-tRNA synthetase  
ROLE: PRP (Properties)  
(methionine- and cysteine-tRNA recognition by, anticodon  
and discriminator base dependence of)

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